channels (LTCC) has been reported in hypertrophy and heart failure. We hypothesize that the altered expression of the $Ca_v\beta$ subunits in cardiac hypertrophy and heart failure results in the altered coupling and regulation of $I_{Ca,T}$. We found an increase in the $Ca_v\beta_1$ and $Ca_v\beta_3$ mRNA and $Ca_v\beta_2$ and $Ca_v\beta_4$ subunit isoform at the protein level in mouse ventricles in a transthoracic aortic constriction (TAC) induced pathological cardiac hypertrophy model. Whole-cell patch clamp electrophysiology using transiently transfected HEK293 cells revealed that co-expression of $Ca_v\beta_1$ or $Ca_v\beta_2$ with $Ca_v3.2$ channel isoform resulted in a significant increase in peak $I_{Cav3.2}$ and a rightward shift in the $V_{1/2}$ of activation and significantly slower inactivation of $I_{Cav3.2}$. On the other hand, co-expression of $Ca_v\beta_3$ or $Ca_v\beta_4$ significantly reduced the peak $I_{Cav3.2}$. In contrast, co-expression of $Ca_v\beta$ isoforms did not alter $I_{Cav3.1}$. Furthermore, co-immunoprecipitation studies in transiently transfected HEK293 cells also demonstrated that the $Ca_v3.2$ channel separately co-immunoprecitated with anti-$Ca_v\beta_1$ or anti-$Ca_v\beta_2$ antibody. In conclusion, our data suggest that the $Ca_v\beta_1$ and $Ca_v\beta_2$ subunits of the LTCC may regulate $I_{Cav3.2}$ in cardiomyocytes during pathological cardiac hypertrophy.

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Homologous Serine/Threonine in the Ca_V 2.2α_1 and 2.3α_1 Subunits Behave Similarly, as Stimulatory and Inhibitory PKC Sites

Ganesan L. Kamatchi.
Biology, Norfolk State University, Norfolk, VA, USA.

High voltage-gated calcium (Cav) channels are regulated by PKC isozymes. These isozymes target selected serine/threonine (Ser/Thr) PKC phosphorylation sites in the intracellular regions of Cavα_1 subunits of these channels. It has been found earlier using Cav2.2α_1 subunits that stimulatory (Thr-422, Ser-2108 and Ser-2132) and inhibitory (Ser-425) PKC sites exist and their activation with PKC isozymes led to potentiation and depression of calcium currents (I_{Ca}) respectively. Based on the above report, it was planned to examine if the homologous sites in the Cav2.3α_1 subunits behave similarly. In this regard the WT Cav2.3α_1 or Ser/Thr Ala mutants of stimulatory (Thr-365, Ser-1995 and Ser-2011) or inhibitory (Ser-369) sites were expressed along with β_{1b} and $_2$/δ cDNA subunits in *Xenopus* oocytes and the barium currents (I_{Ba}) were studied. Intracellular injection of PKC isozymes βII or ε potentiated WT Cav2.3 currents. While both PKC βII and ε potentiated I_{Ba} through Thr-365 (**T365**/S369A/S1995A/S2011A), only PKC ε increased I_{Ba} through Ser-1995 (T365A/S369A/**S1995**/S2011A) channels. Ser-2011 failed to act as a stimulatory site contrary to its homologous site, Ser-2132 in the Cav2.2α_1 subunits. However, Ser-369 acted as inhibitory site as its homolog Ser-425 in the Cav2.2α_1 subunits. Both PKC βII and ε inhibited I_{Ba} through Ser-369 (T365A/**S369**/S1995A/S2011A) channels. When both Thr-365 and Ser-369 were present (**T365/S369**/S1995A/S2011A), I_{Ba} was neither stimulated nor inhibited. However, stimulation was dominant when two stimulatory sites (Thr-365 & Ser-1995) were present along with Ser-369. Experiments with other mutants, including Ser/Thr Asp constructs are being studied and will be discussed.

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Norepinephrine Upregulates T-Type Calcium Channels in Rat Pinealocytes

Haijie Yu[1], Jong Bae Seo[1], Seung-Ryoung Jung[1], Duk-Su Koh[1,2], Bertil Hille[1].
[1]Physiology and Biophysics, University of Washington, Seattle, WA, USA, [2]Physics, Pohang University of Science and Technology, Pohang, Korea, Republic of.

The mammalian pineal has a circadian rhythm of melatonin secretion at night triggered by norepinephrine (NE) released from sympathetic nerve terminals. We asked whether functional expression of voltage-gated calcium channels in rat pinealocytes is changed by culturing them in NE as a surrogate for the night signal. Channel activity was assayed as ionic currents under patch clamp. Cultured without NE, pinealocytes showed only non-inactivating L-type dihydropyridine-sensitive calcium current. After 24 h in NE, an additional low-voltage activated transient calcium current developed whose pharmacology and kinetics corresponded to a T-type channel. This change was initiated by β-adrenergic receptors, cyclic AMP, and protein kinase A as revealed by pharmacological experiments. Quantitative PCR experiments showed mRNA for Cav1.4 (L-type), Cav3.1 and Cav3.2 (both T-type). Cav3.1 T-type channel mRNA was significantly elevated by culture in NE, but those for Cav1.4 and Cav3.2 were not. After only 8 h of NE treatment, Cav3.1 mRNA was already elevated, but the transient calcium current was not. Even a 16 h wait without NE following the 8 h NE treatment induced little additional transient current. However, these cells were primed to make transient current after a second NE exposure. Induction of transient current was sensitive to the inhibitors of intracellular protein trafficking. The NE-induced T-type channel mediated an increased calcium entry during short depolarizations and supported modest transient electrical responses to depolarizing stimuli. Such experiments reveal a potential for circadian regulation of pinealocyte electrical excitability and calcium signaling. This work is supported by National Institutes of Health grants GM-83913, NS-08174 and DK-080840.

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Single-Channel Analysis of the Inhibition of the Calcium Dependent Inactivation by the C-Terminal Modulator Domain of Cav1.3 Channels

Elza Kuzmenkina, Elena Novikova, Wanchana Jangsangthong, Stefan Herzig.
Pharmacology, University of Cologne, Cologne, Germany.

Cav1.3 channels belong to the family of the voltage-gated L-type calcium channels. Because of activation at low voltage thresholds, Cav1.3 channels are involved in regulation of the cell firing but they are also linked to the generation of the oxidative stress. Calcium-dependent inactivation (CDI) is a negative feedback process terminating the potentially toxic calcium influx (Johny et al., Nat Commun 2013;4:1717). Multiple Cav1.3 splice isoforms, which can be simultaneously expressed in the same tissue, differ in their channel gating and CDI (Bock et al., J Biol Cem 2011;286:42736).

We applied single-channel patch-clamp measurements to compare two native isoforms with alternatively spliced C-terminus. Cav1.3 42 isoform has a long C-terminus containing C-terminal modulator domain (CTM), whereas in the short C-terminus of Cav1.3 42A isoform CTM is truncated. CTM shifts channel activation to higher voltages and inhibits CDI.

Here, we observed that calcium influx from a few single-channel openings lead to CDI, visible as a decay of the average single-channel current. However, the extent of CDI was significantly reduced in Cav1.3 42 isoform as compared with 42A isoform. Furthermore, we observed the shortening of the open times as a result of CDI (Imredy and Yue, Neuron 1994;12:301). The degree of the open-time shortening was dependent on the prior calcium influx (Josephson et al., J Physiol 2010;588:213) with a steeper dependence for Cav1.3 42A isoform.

Additionally, we performed experiments with a channel agonist S(-)BayK 8644. The boosting of the channel activity by BayK 8644 eliminated the difference between the isoforms, resulted in a high extent of CDI for both splice variants.

Our findings suggest the ability of CTM to inhibit CDI depends on the channel activity, which can be in turn modified by CTM.

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Serum Factor Alters T-Type Cav3.2 Gating Kinetics and Current Density

Gray Evans, Slobodan M. Todorovic.
Department of Anesthesiology, University of Virginia, Charlottesville, VA, USA.

T-type Calcium channels play a critical role in regulating neuronal excitability and modulating sensory transmission. The Cav3.2 channel isoform is highly expressed in peripheral nociceptors as well as in the pain-processing regions of the dorsal horn of the spinal cord. Augmenting Cav3.2 currents has been shown to induce hyperexcitability in nociceptive neurons in vitro and hyperalgesia in vivo. These studies strongly suggest that potentiation of the Cav3.2 channel results in abnormal nociceptive transmission, which could contribute to a variety of clinical pain syndromes. Therefore, it is important to identify endogenously produced molecular species that modulate Cav3.2 currents. Using the patch-clamp technique and stably transfected human embryonic kidney cells (HEK-293) expressing the Cav3.2 channel, we have begun to characterize a factor found in fetal bovine serum (FBS) that profoundly affects Cav3.2 channel gating kinetics. Specifically, when compared to baseline recombinant currents, 1% serum produces maximal increases in current magnitude (350%; $p<.001$), conductance (150%, $p<.001$), rate of macroscopic inactivation (47.1%; $p<.001$) and deactivation (74.3%; $p<.001$). Furthermore, 1% serum induces a hyperpolarizing shift in voltage-dependence of activation (V50) (−4.77mV; $p<.001$) with minimal effect on voltage-dependence of inactivation. In contrast, we found that recombinant CaV3.1 currents were completely insensitive. Similar to recombinant Cav3.2 currents, T-currents from rat dorsal root ganglia (DRG) cells exhibited comparable changes, with 1% serum producing maximal increases in current magnitude (210%; $p<.05$), conductance (183%; $p<.05$), and rate of inactivation (71.4%; $p<.001$), in addition to inducing a hyperpolarizing shift in V50 (−9.402mV; $p<.01$). Future studies will focus on identifying this serum factor in order to evaluate its potential role in nociceptive signal modulation and cellular excitability. Supported in part by NIH grant R21DA034448 (SMT).